



EXAMPLE 1

Solid Phase Peptide Synthesis

Solid phase peptide synthesis (SPPS) was carried out on a 0.25 millimole (mmole) scale using an Applied Biosystems Model 431A Peptide Synthesizer and using 9-fluorenylmethyloxycarbonyl (Fmoc) amino-terminus protection, coupling with dicyclohexylcarbodiimide/hydroxybenzotriazole or 2-(1H-benzo-triazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/ hydroxybenzotriazole (HBTU/HOBT), and using p-hydroxymethylphenoxy-methylpolystyrene (HMP) or Sasrin™ resin for carboxyl-terminus acids or Rink amide resin for carboxyl-terminus amides.

Homocysteine (Hcy) was prepared by alkaline hydrolysis of L-homocysteine lactone or by reduction of homocystine using metallic sodium in liquid ammonia. Fmoc.Hcy(S-trityl) and Fmoc.Pen(S-trityl) were prepared from the appropriate precursor amino acids by tritylation with triphenylmethanol in trifluoroacetic acid, followed by Fmoc derivitization as described by Atherton et al. (1989, Solid Phase Peptide Synthesis, IRL Press; Oxford). 4piperidinyl butyl ether derivatives of tyrosine (Y[(CH₂)₄-piperidine]) were prepared by SPPS butyl starting with Fmoc-tyrosine-(4-Boc-piperidine ether). Fmoc-S-(3-Bocaminopropyl)cysteine was prepared from L-cysteine and Boc-aminopropyl bromide in methanolic sodium methoxide followed by treatment with O-9-fluorenylmethyl-O'-Nsucceinimidyl carbonate (FmocOSu) at pH 10. 4-amidinophenylalanine (Amp) was prepared as described in co-owned and co-pending PCT International Patent Application Serial No. PCT/US94/03878, incorporated by reference.

Where appropriate, 2-haloacetyl groups were introduced either by using the appropriate 2-haloacetic acid as the last residue to be coupled during SPPS or by treating the N-terminal free amino group of the peptide bound to the resin with either 2-haloacetic acid/diisopropylcarbodiimide/ N-hydroxysuccinimide in NMP or 2-halo-acetic anhydride/diisopropylethylamine in NMP.

Where appropriate, 2-haloacetylated peptides were cyclized by stirring an 0.1 - 1.0 mg/mL solution in phosphate or bicarbonate buffer or dilute ammonium hydroxide (pH 8) containing 0.5 - 1.0 mM EDTA for 4 - 48 hours, followed by acidification with acetic acid,

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